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Report of the fifth interlaboratory comparison organised by the Community Reference Laboratory for Heavy Metals in Feed and Food

Total Cd, Pb and As and extractable Cd and Pb in mineral feed

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Heavy Metals
in Feed and Food

Report of the fifth interlaboratory comparison

Total Cd, Pb and As, and extractable Cd and Pb in mineral feed

IMEP®

January 2009

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1 Summary

The Institute for Reference Materials and Measurements (IRMM) of the Joint Research Centre, a Directorate-General of the European Commission, operates the Community Reference Laboratory for Heavy Metals in Feed and Food (CRL-HM). One of its core tasks is to organise interlaboratory comparisons (ILCs) among appointed National Reference Laboratories (NRLs). This report presents the results of the fifth ILC of the CRL-HM which focused on the determination of total Cd, Pb and As and extractable Cd and Pb in mineral feed according to Directive 2002/32/EC[1] of the European Parliament and of the Council on undesirable substances in animal feed.

The test material used in this exercise was a commercial mineral feed for piglets provided by AGES, Zentrum Analytik und Mikrobiologie, in Austria. The material, naturally contaminated, was processed, bottled, labelled and dispatched by the Reference Materials Unit of the IRMM. The samples were dispatched on the second half of October 2008. Each participant received one bottle containing approximately 30 g of test material. Thirty-one participants from 25 countries registered to the exercise of which 29 submitted results for total Cd and for total Pb, 22 submitted results for total As and 27 submitted results for extractable Cd and for extractable Pb. Two laboratories did not submit results due to a break down in the instruments that were to be used for the analyses.

The assigned values (X_{ref}) for total and extractable Cd and Pb were provided by IRMM using isotope dilution-inductively coupled plasma-mass spectrometry (ID-ICP-MS). The analytical uncertainty of X_{ref}, u_{char}, was calculated according to the ISO Guide to the Expression of Uncertainty in Measurement (GUM)[2]. The assigned value for total arsenic was provided by the Studiecentrum voor Kernenergie (SCK-CEN) using neutron activation analysis. The analytical uncertainty of X_{ref}, u_{char}, for total arsenic was calculated according to GUM[3,4]. Homogeneity and stability studies were subcontracted to Bayer Antwerpen. The uncertainties of the respective assigned values, u_{ref}, were calculated combining the analytical uncertainty, u_{char}, with a contribution for the between-bottle homogeneity, u_{bb}, and for the short term stability of the test material, u_{sts}. Participants were invited to report the uncertainty of their measurements. This was done by 25 laboratories for total Cd, 24 laboratories for total Pb, 18 laboratories for total As, 22 laboratories for extractable Cd and 21 laboratories for extractable Pb.

The laboratory performance was evaluated using z and zeta scores in accordance with ISO 13528[5]. The standard deviations for proficiency assessment (also called target standard deviation) were calculated using the modified Horwitz equation[6] and were between 15 and 16 % for all the measurands .

2 Introduction

The basic nutrients that animals require for growth, reproduction, and good health include carbohydrates, proteins, fat, vitamins and minerals. Minerals essential for animal life include sodium chloride, calcium, phosphorus, sulphur, potassium, magnesium, manganese, iron, copper, cobalt, iodine, zinc, molybdenum, and selenium. The last six being toxic when provided in excessive amounts.

All farm animals generally need more sodium chloride than is contained in their feed, they are supplied with it regularly. Of the other essential minerals, phosphorus and calcium are most

apt to be lacking, because they are heavily drawn upon to produce bones, milk, and egg shells. Phosphorous supplements are bone meal, dicalcium phosphate, and defluorinated phosphates. Egg shells are nearly pure calcium carbonate. Calcium may readily be supplied by ground limestone, ground shells or marl that is high in calcium.
Small amounts of iodine are needed by animals for the formation of thyroxine. A serious deficiency of iodine may cause goitre, a disease which has caused in certain regions heavy losses of newborn pigs, lambs, kids, calves, and foals.

In some areas, soil and forage are deficient in copper and cobalt, which are needed together with iron for the formation of haemoglobin. In these areas, farm animals may suffer from anaemia unless the deficiency is corrected by suitable mineral supplement.

Iron, is amply supplied in most animal feed, except milk. The only practical problem with iron deficiency occurs in young suckling pigs before they start to consume other feed in addition to milk.

Though manganese is essential for animals, the usual rations for all farm animals, except poultry, supply sufficient quantities. A lack of manganese can cause the nutritional disease of chicks and young turkeys called *perosis* and may also cause failure of eggs to hatch.

Normal rations for swine are often deficient in zinc, especially in the presence of excess calcium. Adding 100 parts per million of zinc carbonate cures zinc deficiency symptoms, which include retarded growth and severe scaliness and cracking of the skin.

A trace of selenium is necessary for normal health of animals; excessive amounts found in forages in some regions poison animals may cause death.

To furnish both calcium and phosphorus, livestock may be feed a mixture of 60 % dicalcium phosphate and 40 % sodium chloride.

To overcome problems associated with a high metal content in feed, maximum levels for trace elements in several commodities have been laid down in Directive 2002/32/EC, and a network has been built up to ensure quality and comparability in official controls throughout the European Union[7]. In March 2006 a footnote was introduced in Directive 2002/32/EC in which it is stated that *"Maximum levels refer to an analytical determination of lead and cadmium, whereby extraction is performed in nitric acid 5 % (W/W) for 30 minutes at boiling temperature"*.

In the second half of 2007 the CRL-HM organised a proficiency test (PT) exercise (IMEP-103) for the network of appointed NRLs to determine total and extractable Cd and Pb in compound feed for fish. The outcome of that exercise was that total and extractable Cd concentrations in that test material were identical. The Youden plots constructed with participant results for total and extractable Pb, indicated no method dependence although more studies are needed before extracting any definitive conclusion on the Pb matter due to a lack of homogeneity for Pb in the test material.

With the aim of expanding the previously mentioned study to a wider variety of feed matrices, the CRL-HM has organised a PT for the determination of total Cd, Pb and As and extractable Cd and Pb in mineral feed. The two latter measurands were to be determined using the same extraction procedure as in IMEP-103 which was agreed upon by the CRL-HM and the

network of NRLs, and which is agreement with the requirements laid down in Directive 2002/32/EC.

3 Scope

As stated in Regulation 882/2004 of the European Parliament and of the Council[8], one of the core duties of the CRL-HM is to organise interlaboratory comparisons for the benefit of staff from National Reference Laboratories. The scope of this ILC is to test the competence of the appointed NRLs to determine the total concentration of Cd, Pb and As and of extractable Cd and Pb according to Directive 2002/32/EC.

The assessment of the measurement results is undertaken on the basis of requirements laid down in legislation[1], and follows the administrative and logistic procedures of IMEP, the International Measurement Evaluation Program of the IRMM of the European Commission Directorate Joint Research Centre. IMEP is accredited according to ISO Guide 43. The designation of this ILC is IMEP-105.

4 Time frame

The interlaboratory comparison was agreed upon by the NRL network at the second CRL-HM workshop held on 24/25 September 2007. Specific details of the exercise were refined during the third CRL-HM workshop held on 25/26 September 2008. Invitation letters were sent to the participants on 3rd October 2008 (cf. Annex 1). The samples were dispatched to the participants on 20th October 2008. Reporting deadline was 30th November 2008.

5 Test material

5.1 Preparation

The test material, commercially available mineral feed for piglets, was provided by AGES, Zentrum Analytik und Mikrobiologie. Upon arrival at IRMM the material was processed by the Reference Materials Unit as follows: The material was milled to obtain particles around 500 µm with a Retsch, Heavy Duty mill. The particle size distribution was assessed by laser diffraction and the water content determined by Karl-Fisher titration. Coarse particles were removed sieving through a 500 µm sieve. The material was then homogeneised and distributed using a vibrating feeder into amber glass bottles (60 mL) with polyethylene (PE) insert and screw cap lid with crimp film, containing approximately 30 g of test material each. Before processing the material was stored at room temperature. After processing the storing temperature was 4 ºC. Processing took place at room temperature.

5.2 Homogeneity and stability

The measurements for the homogeneity and stability studies were performed by the Central Laboratory of Bayer Antwerpen. Homogeneity was evaluated according to ISO 13528 and to the method proposed by Fearn and Thompson[9] (one of the approaches recommended by the IUPAC International Harmonised Protocol[10]). Homogeneity and stability studies were performed for extractable Cd and Pb. Our past experience showed that total and extractable Cd and Pb behave the same in terms of homogeneity and stability. The material proved to be

homogeneous according to the IUPAC International Harmonised Protocol and to ISO 13528 for total As and for extractable Cd. It was hence assumed that the material was also homogeneous for total Cd. For Extractable Pb, the material was not homogeneous neither according to the IUPAC International Harmonised Protocol nor to ISO 13528, and it was therefore concluded that total Pb was also not homogeneous. Figure 1 shows that the material consisted of a mixture of materials characterised by different colours. It was decided not to mill the material down to finer powder in order to keep the test material similar to real routine samples. For this reason, u_{bb} was set to 10 % as provided by the software SoftCRM[11], and propagated according to GUM.

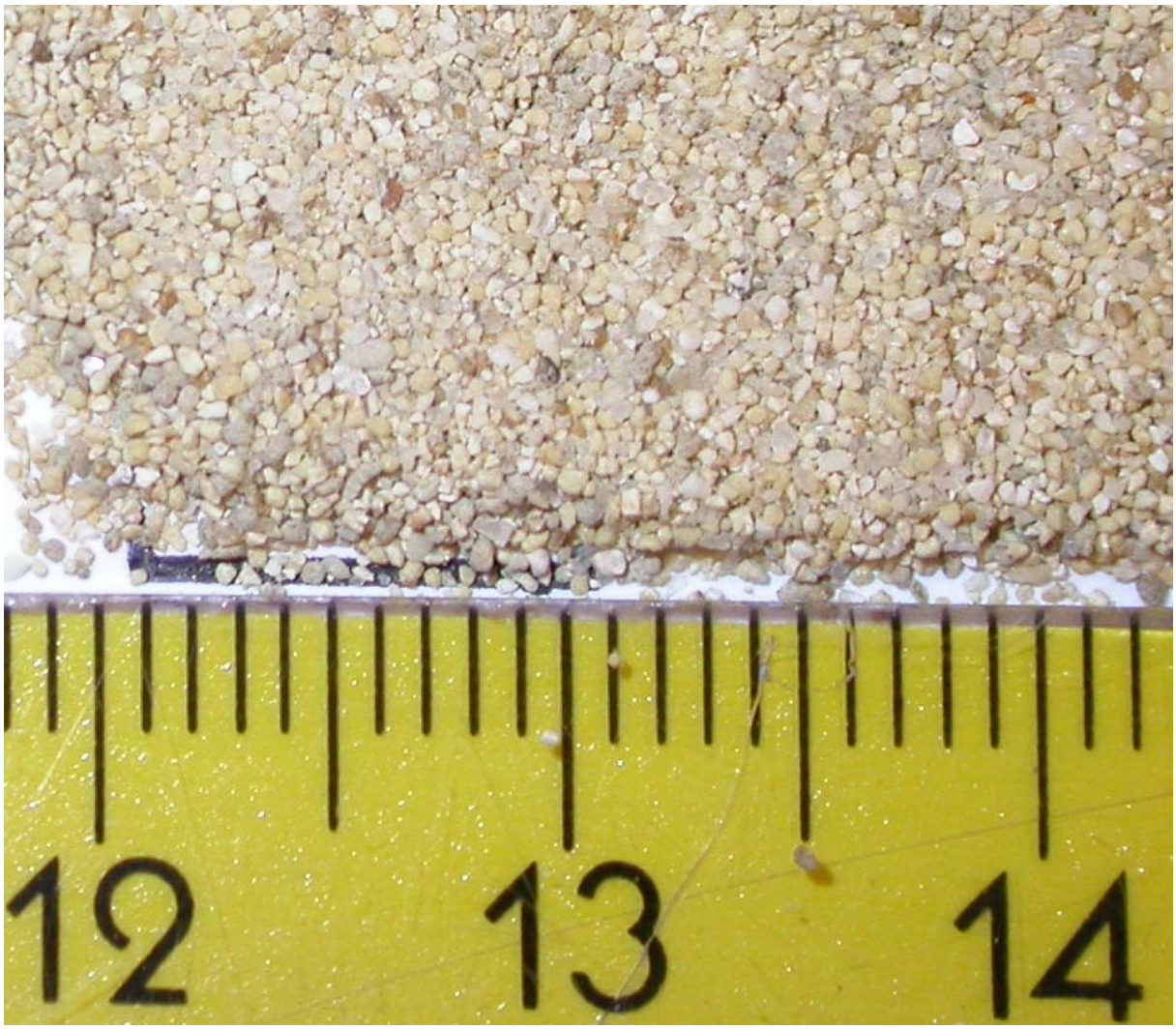

Figure 1: Picture of the test material used in IMEP-105.

The stability study of the test material was conducted following the isochronous approach[12]. The evaluation of the stability of the test material was made using the software SoftCRM[13]. The material proved to be stable at room temperature for the six weeks that elapsed between the dispatch of the samples and the deadline for submission of results for all the tested measurands. The results for Pb were highly scattered, confirming the lack of homogeneity observed, earlier described. u_{sts} was therefore set to zero, to avoid overestimation of the uncertainty.

The analytical results and statistical evaluation of the homogeneity and short term stability studies are provided in Annex 2.

5.3 Distribution

One set of material was sent to every participant. The test material was dispatched to the participants by IRMM on 20th October 2008. Each participant received: a) one bottle containing approximately 30 g of test material*, b) an accompanying letter with instructions on sample handling and reporting and with the method to be applied for the determination of extractable Cd and Pb (cf. Annex 3) and c) a form which had to be sent back after receipt of the sample to confirm its arrival (cf. Annex 4).

6 Instructions to participants

Details on this ILC were discussed with the NRLs at the second workshop organised by the CRL-HM in September 2007. Concrete instructions were given to all participants in a letter that accompanied the samples (Annex 3). The measurands and matrix were clearly defined as *"Total Cd, Pb and As and extractable amounts of Cd and Pb in mineral feed"*.

Laboratories were asked to perform two or three independent measurements and report them, together with the mean of the results and its associated uncertainty. Some laboratories reported four independent results. Participants were asked to follow their routine procedures for the determination of total Cd, Pb and As and the procedure previously agreed upon for the determination of extractable Cd and Pb. The results were to be reported in the same manner (e.g. number of significant figures) as when reporting to customers.

The results were to be reported in a special on-line form for which every participant received an individual access code. A special questionnaire, aiming at collecting additional information, was included in the online form. The questionnaire is presented in Annex 5.

7 Reference values an their standard uncertainties

The reference values, X_{ref}, for this ILC for total and extractable Cd and Pb were determined by IRMM using Isotope Dilution Inductively Coupled Plasma (ID-ICP-MS). IRMM has proven its measurement capabilities by successful participation in the Comité Consultative de la Quantité de Matière (CCQM) key comparisons. For total As the reference value was provided by the Studiecentrum voor Kernenergie (SCK-CEN) using neutron activation analysis. SCK has participated in key comparisons organised by the CCQM for the determination of total arsenic in different matrices, with satisfactory results.

The standard uncertainty associated to the assigned value (u_{ref}) was calculated as:

$$u_{ref} = \sqrt{u_{char}^2 + u_{bb}^2 + u_{sts}^2} \qquad \text{Eq. 1}$$

* Two laboratories received two bottles because the method to be used, dry ashing, required the use of 5 g of material per replicate and 30 g would not be enough for the five measurands plus the water content determination

Where:
u_{ref}: standard uncertainty associated to the assigned value
u_{char}: standard uncertainty of characterisation
u_{bb}: standard uncertainty contribution for the between-bottle homogeneity
u_{sts}: standard uncertainty contribution derived from the short-term stability study

The values of X_{ref}, u_{char}, u_{bb}, u_{sts}, u_{ref} and the expanded standard uncertainty U_{ref}, are summarised in Table 1.

Table 1: assigned values and their standard uncertainties for the measurands of this ILC.

	X_{ref} [mg kg^{-1}]	u_{char} [%]	u_{bb} [%]	u_{sts}* [%]	u_{ref} [%]	U_{ref} [%]
Total Pb	1.65	5.8	10	0 #	12	23
Extract. Pb	1.29	5.4	10	0 #	11	23
Total Cd	1.71	1.6	2	2.1	3.3	6.7
Extract. Cd	1.71	1.6	2	2.1	3.3	6.7
Total As	1.18	3.8	2	3.8	5.7	12

***** For six weeks
# See section 5.2
X_{ref} is the certified reference value and uref the corresponding standard uncertainty; U_{ref} is the estimated expanded uncertainty, with a coverage factor k=2, corresponding to a level of confidence of about 95%.

As summarised in Table 1, total digestion and partial extraction of the test material, following the procedure described in the accompanying letter to the participants, provide identical Cd concentrations. This finding is supported by the Youden plot, Figure 2.a, constructed with the results provide by the participants in this exercise. One single cloud of points is observed on both axes around the reference value and within the square formed by the reference values ± sigma-hat, when total vs extractable Cd is plotted, showing that the results are not dependent of the method applied. With the exception of one participant, the laboratories that have deviated in their reported values from the reference values, show the same bias in both the total and the extractable Cd, and so they are spread along the diagonal of the Youden plot.

In the case of Pb, the reference value obtained by IRMM for extractable Pb is about 80% of the total Pb concentration. In the Youden Plot, Figure 2.b it can be seen that most results were negatively biased, and that most laboratories reported concentrations lower than the reference values both for the total and the extractable Pb. Nevertheless, for most laboratories the bias seems to be higher in the results reported for total Pb than for extractable Pb, so that the values are not spread along the diagonal of the Youden Plot but along a line which matches quite well a parallel to the Youden Plot diagonal.

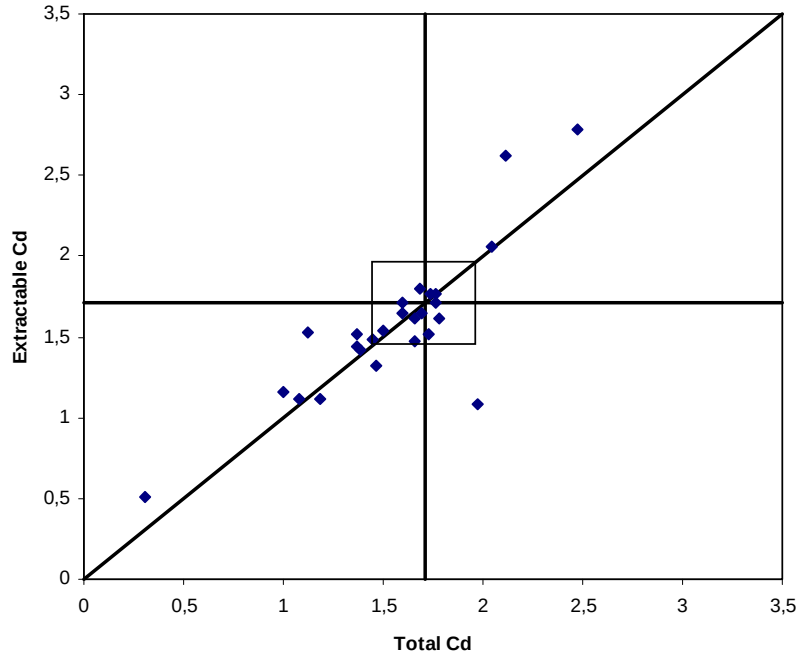

Figure 2.a
Youden plot for total and extractable Cd as obtained from the participants.

in mg kg^{-1}

_____ X_{ref}

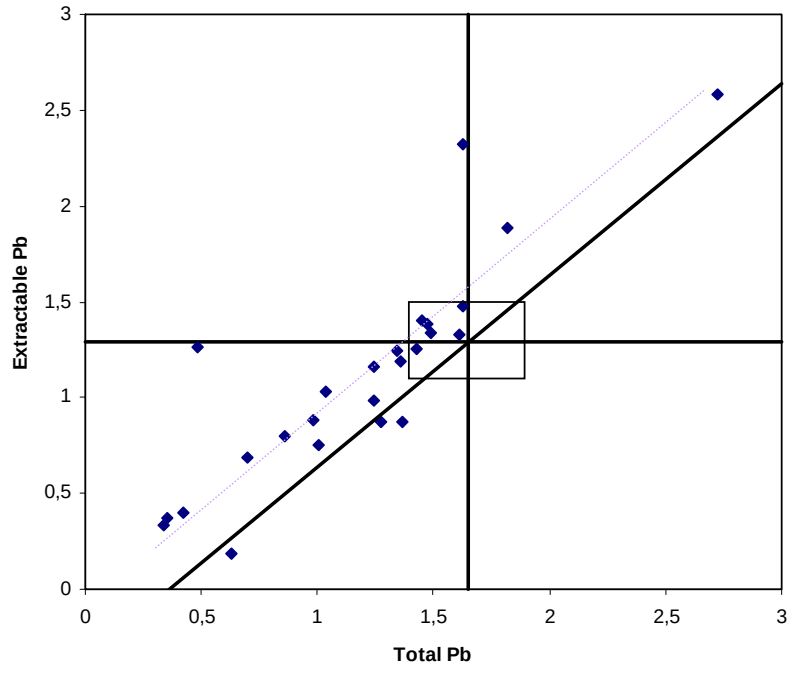

Figure 2.b
Youden plot for total and extractable Pb as obtained from the participants.

in mg kg^{-1}

_____ X_{ref}

8 Evaluation of results

8.1 General observations

Thirty-one participants from 25 countries registered to the exercise of which 28 submitted results for total Cd and for total Pb (one out of the 28 reported "< than" for total Pb), 22 submitted results for total As and 27 submitted results for extractable Cd and for extractable Pb (one out of the 27 reported "< than" for extractable Pb).

Two laboratories did not submit results due to a break down in the instruments that were to be used for the analyses. Twenty-five laboratories reported uncertainty for total Cd, 24 for total Pb, 18 for total As, 22 for extractable Cd and 21 for extractable Pb. All laboratories responded to the questionnaire included in the online reporting form.

8.2 Scores and evaluation criteria

Individual laboratory performance is expressed in terms of z and zeta scores in accordance with ISO 13528[5].

$$z = \frac{x_{lab} - X_{ref}}{\sigma} \qquad \text{Eq. 2}$$

$$zeta = \frac{x_{lab} - X_{ref}}{\sqrt{u_{ref}^2 + u_{lab}^2}} \qquad \text{Eq. 3}$$

Where:

x_{lab} is the mean of the individual measurement results calculated by the ILC organiser
X_{ref} is the certified reference value (assigned value)
u_{ref} is the standard uncertainty of the reference value
u_{lab} is the standard uncertainty reported by a participant
σ is the standard deviation for proficiency assessment

The z score compares the participant's deviation from the reference value with the standard deviation accepted for the proficiency test, σ. Usually, in the area of food and feed σ is derived from the Thompson improved Horwitz equation[6]. The values for σ obtained for this exercise were 15 % of the assigned value for total and extractable Cd, 15 % for total Pb and 15.5 % for extractable Pb and for total As. If those reproducibilities are considered as satisfactory, the z-score can be interpreted as:

$\|z\| \leq 2$	satisfactory result
$2 < \|z\| \leq 3$	questionable result
$\|z\| > 3$	unsatisfactory result

Zeta score states if the laboratory result agrees with the assigned value within the respective uncertainties. The interpretation of the zeta score is similar to the interpretation of the z-score:

$\|z\| \leq 2$	satisfactory result
$2 < \|z\| \leq 3$	questionable result
$\|z\| > 3$	unsatisfactory result

An unsatisfactory zeta-score might be due to an underestimation of the uncertainty, or to a large error causing a large deviation from the reference value, or to a combination of the two factors. A laboratory with an unsatisfactory zeta-score has an estimation of the uncertainty of its measurements which is not consistent with laboratory's deviation from the reference value. The standard uncertainty should fall in a range between a minimal required (u_{min}), and a maximal allowed (u_{max}) reported standard uncertainty. u_{min} is set to the standard uncertainty of the reference value. It is unlikely that a laboratory carrying the analysis on a routine basis is able to measure the measurand with a smaller uncertainty that the reference laboratory itself. u_{max} is set to the standard deviation accepted for the proficiency test, σ. If the standard

uncertainty from the laboratory, $u_{lab} < u_{min}$ it is likely that the laboratory has underestimated its uncertainty. If $u_{lab} > u_{max}$, some effort should be made to reduce it because it exceeds the present state-of-the-art in that field of analysis. If zeta >2, the results disagree within the expanded uncertainties, if the k-factor is chosen so that the expanded uncertainty is 95 %.

The standard uncertainty of the laboratory (u_{lab}) was calculated dividing the reported expanded uncertainty by the reported coverage factor (*k*). When no uncertainty was reported, it was set to zero ($u_{lab} = 0$). When *k* was not specified, the reported expanded uncertainty was considered as the half-width of a rectangular distribution; u_{lab} was then calculated by dividing this half-width by √3, as recommended by Eurachem and CITAC[14].

Should participants feel that the σ values are not fit for their purpose they can recalculate their scorings with a standard deviation matching their requirements, as recommended in IUPAC.

8.3 Laboratory results and scorings

The results, as reported by the participants, are summarised in Table 2a-e for total Cd, total Pb, total As, extractable Cd and extractable Pb, respectively, together with the z- and zeta scores. Laboratory codes were given randomly.

Three sets of figures are provided for total Cd, extractable Cd, total Pb, extractable Pb and total As (Fig 3-7). Each set includes (a) the Kernel Density plot, (b) individual mean value and associated expanded uncertainty, (c) the z-and zeta scores. The solid line represents the assigned value, the dashed lines delimit the reference interval ($X_{ref} \pm 2u_{ref}$) and the dotted lines delimit the target interval ($X_{ref} \pm 2\sigma$). The Kernel plots were obtained sing a software tool developed by AMC[15]

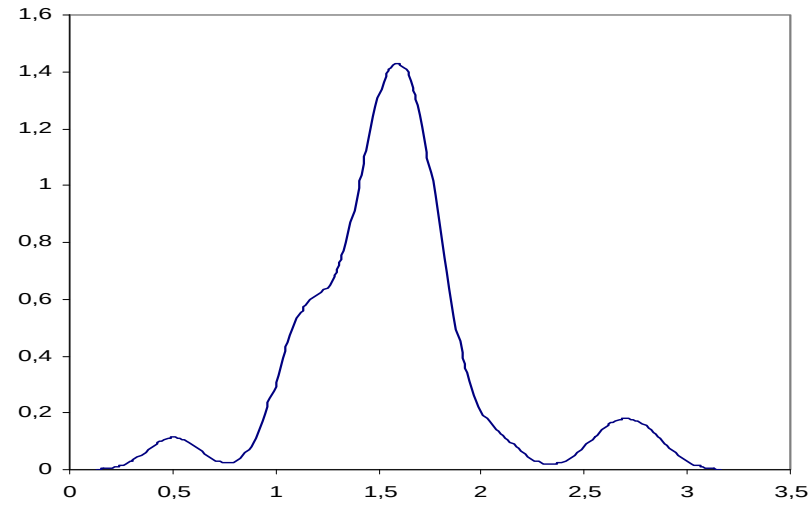

Figure 3a
Total Cd in mineral feed
Kernel density,
all results

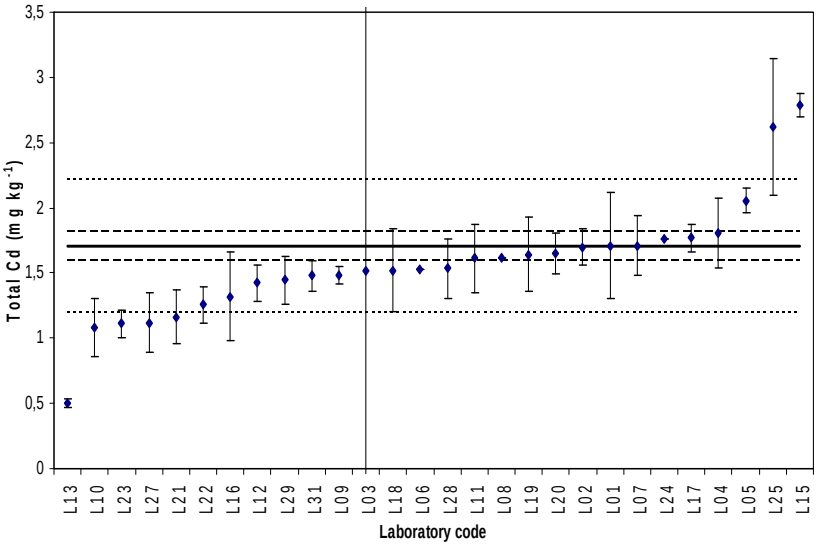

Figure 3b
Reported results and corresponding expanded uncertainties

$X_{ref} = 1.708$
$u_{ref} = 0.057$
$\hat{\sigma} = 0.256$ (15 %)
in mg kg^{-1}

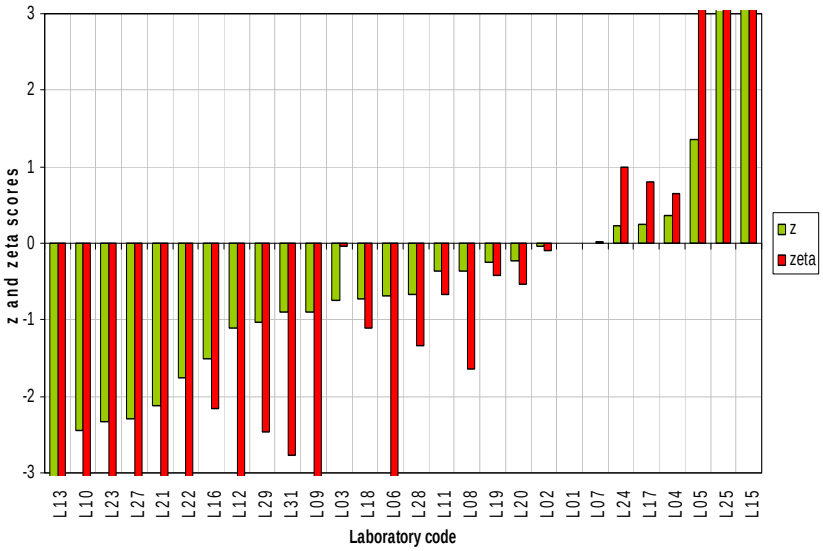

Figure 3c
Performance evaluation
$z = (x_{lab}-X_{ref})/ \hat{\sigma}$
$zeta = (x_{lab}-X_{ref})/ \sqrt{(u_{lab}^2+u_{ref}^2)}$

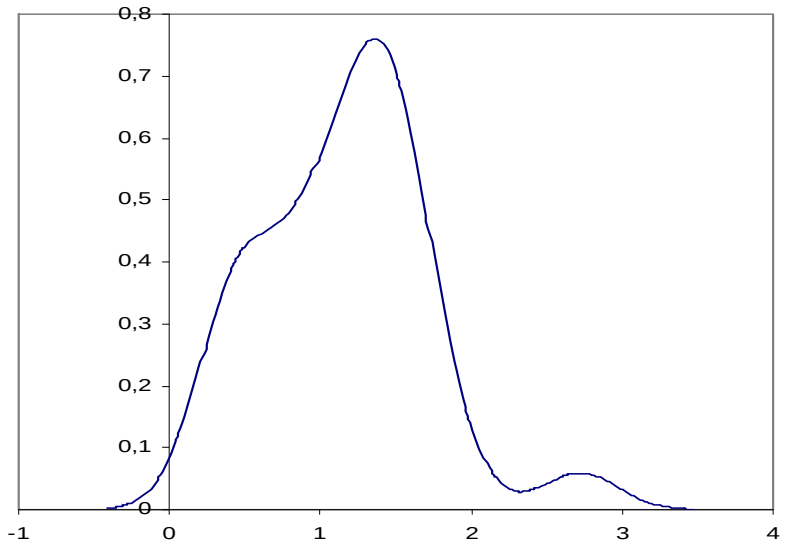

Figure 4a
Total Pb in mineral feed
Kernel density,
all results

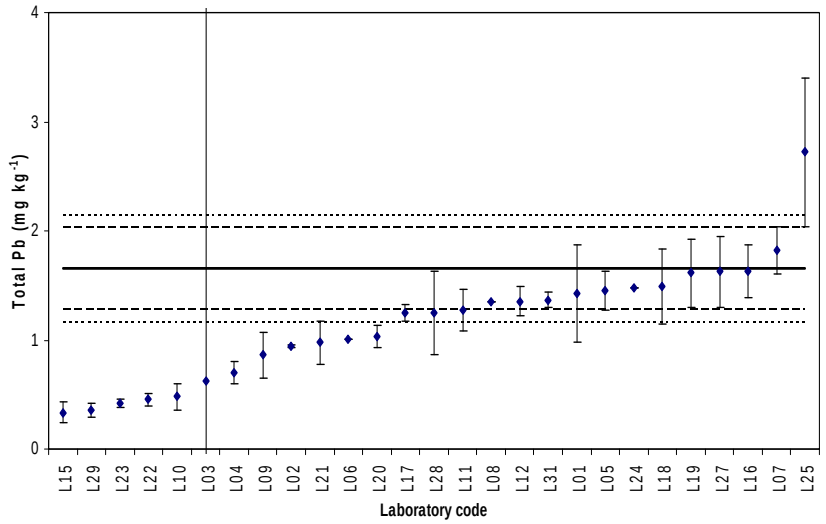

Figure 4b
Reported results and corresponding expanded uncertainties

$X_{ref} = 1.65$
$u_{ref} = 0.19$
$\hat{\sigma} = 0.25$ (15 %)

in mg kg^{-1}

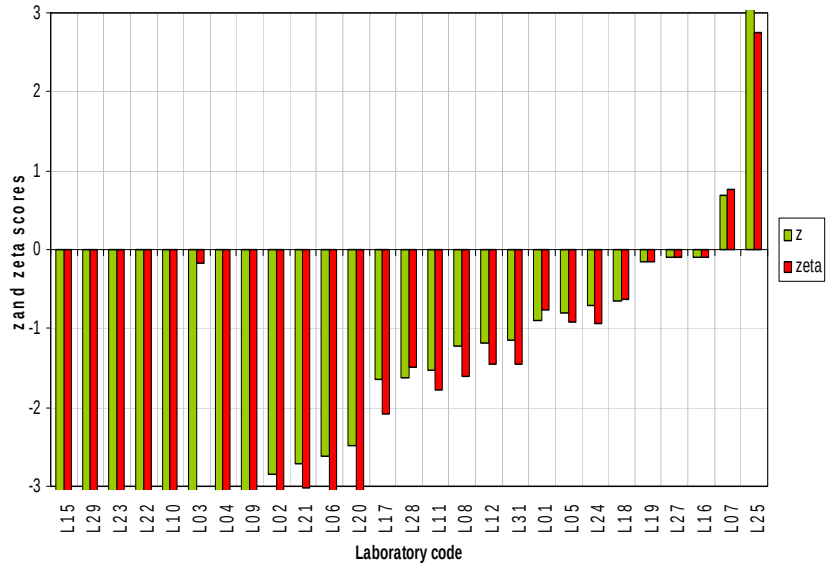

Figure 4c
Performance evaluation
$z = (x_{lab}-X_{ref})/ \hat{\sigma}$
$zeta = (x_{lab}-X_{ref})/ \sqrt{(u_{lab}^2+u_{ref}^2)}$

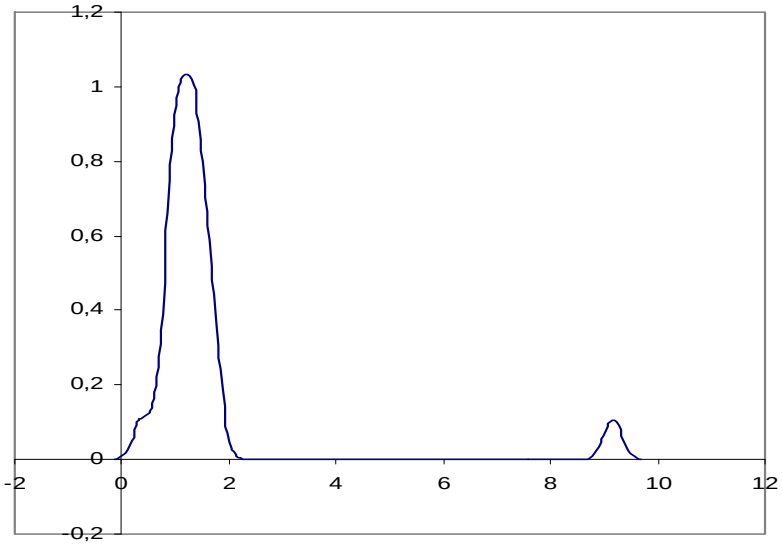

Figure 5a
Total As in mineral feed
Kernel density,
all results

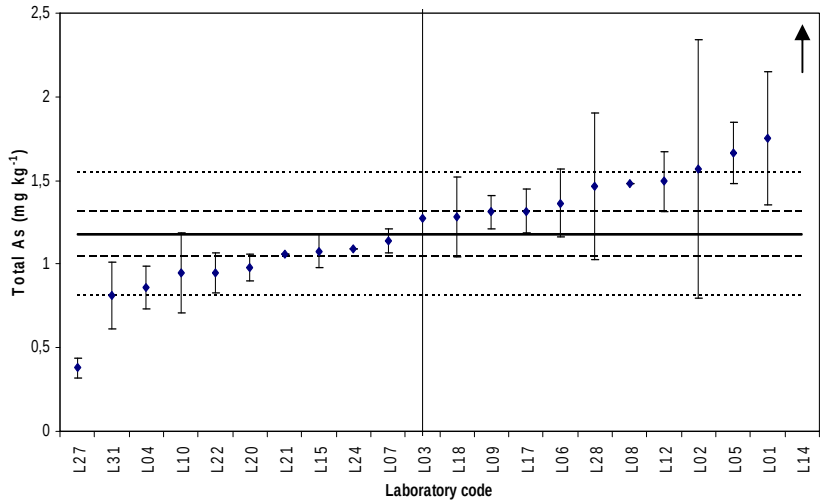

Figure 5b
Reported results and corresponding expanded uncertainties

X_{ref} = 1.18
u_{ref} = 0.09
$\hat{\sigma}$ = 0.18 (15.5 %)
in mg kg^{-1}

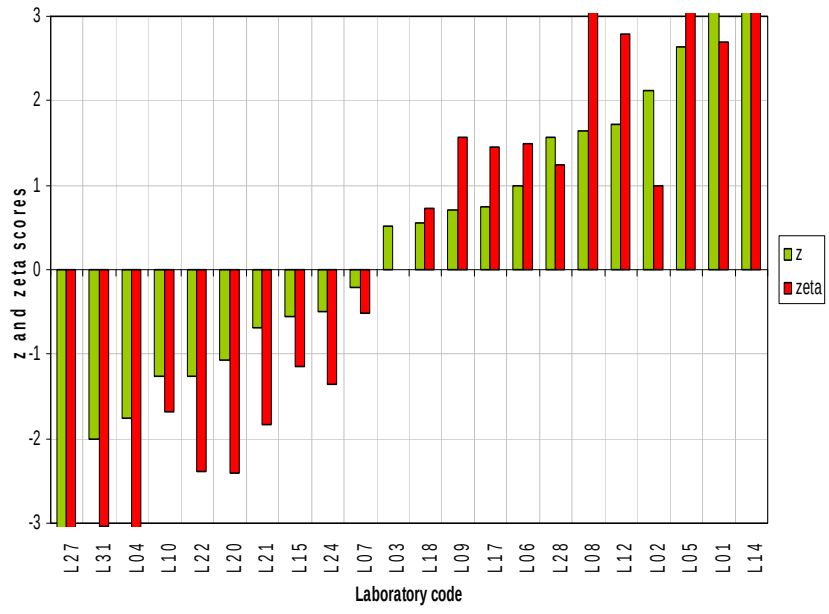

Figure 5c
Performance evaluation
$z = (x_{lab}-X_{ref})/ \hat{\sigma}$
$zeta = (x_{lab}-X_{ref})/ \sqrt{(u_{lab}^2+u_{ref}^2)}$

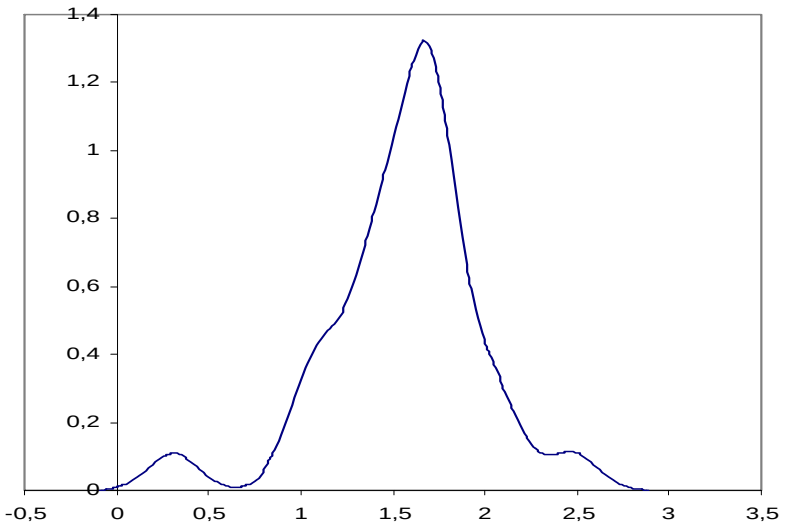

Figure 6a
Extractable Cd in mineral feed
Kernel density,
all results

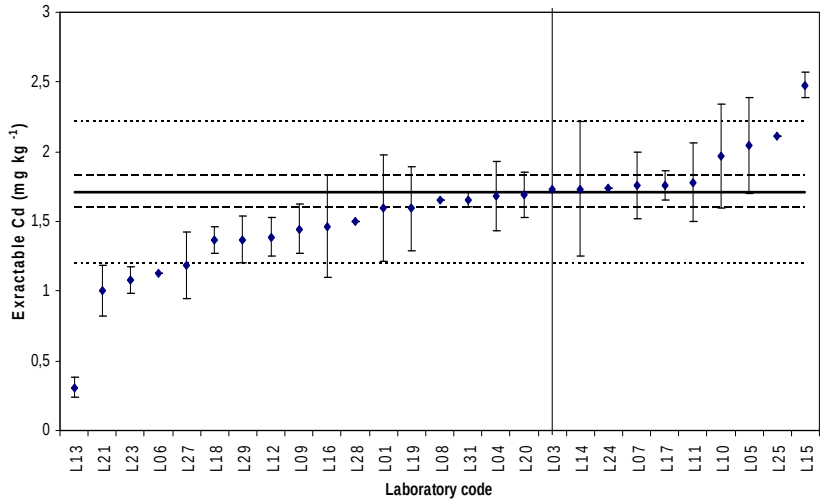

Figure 6b
Reported results and corresponding expanded uncertainties

X_{ref} = 1.708
u_{ref} = 0.057
$\hat{\sigma}$ = 0.256 (15 %)
in mg kg^{-1}

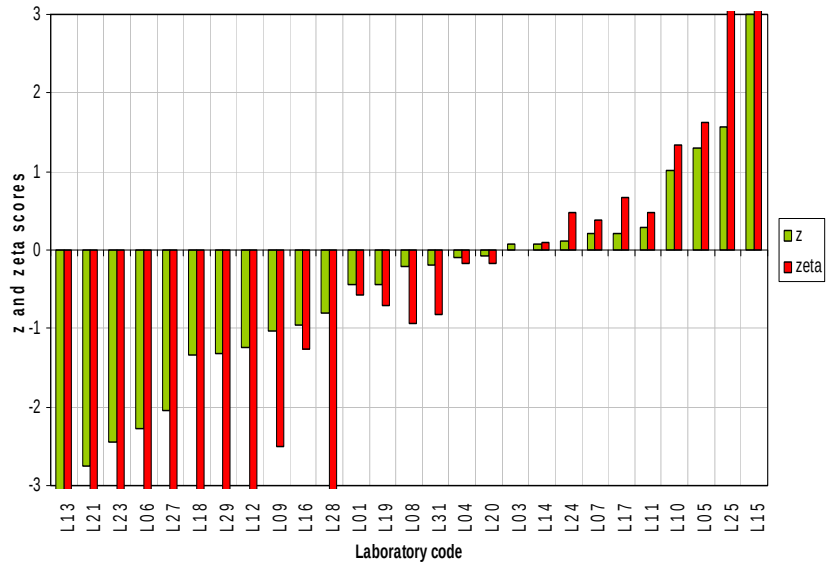

Figure 6c
Performance evaluation
$z = (x_{lab}-X_{ref})/ \hat{\sigma}$
$zeta = (x_{lab}-X_{ref})/ \sqrt{(u_{lab}^2+u_{ref}^2)}$

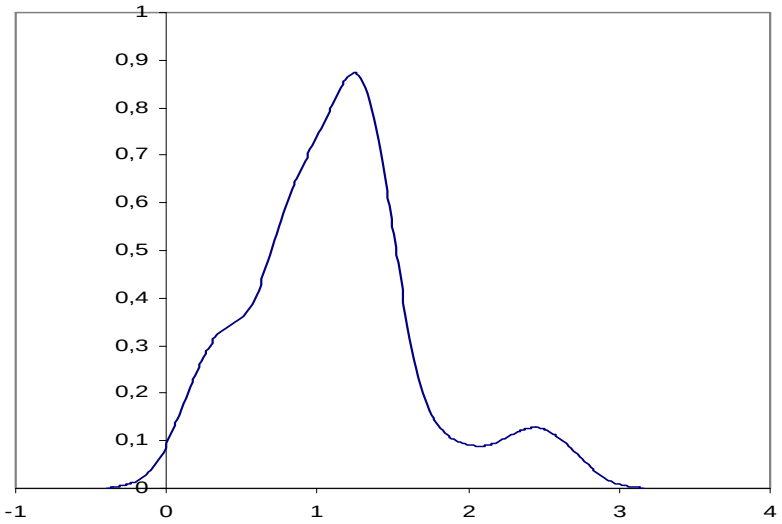

Figure 7a
Extractable Pb in mineral feed
Kernel density,
all results

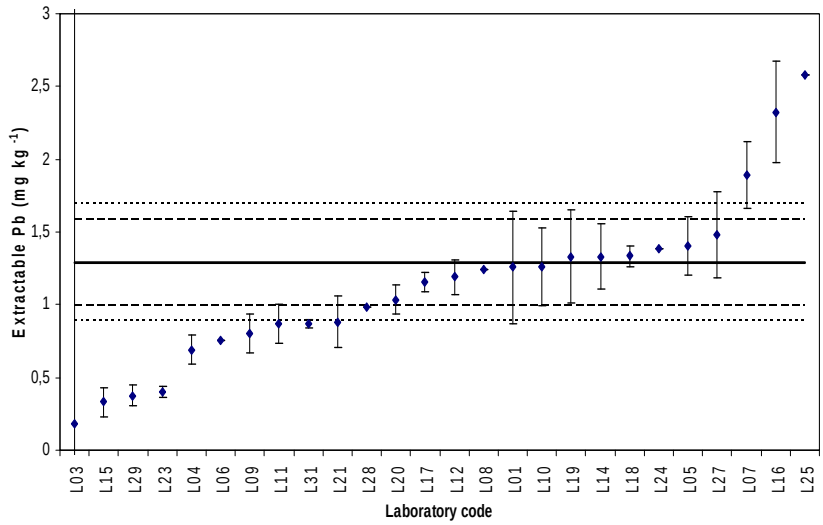

Figure 7b
Reported results and corresponding expanded uncertainties

X_{ref} = 1.29
u_{ref} = 0.14
$\hat{\sigma}$ = 0.20 (15.5 %)
in mg kg^{-1}

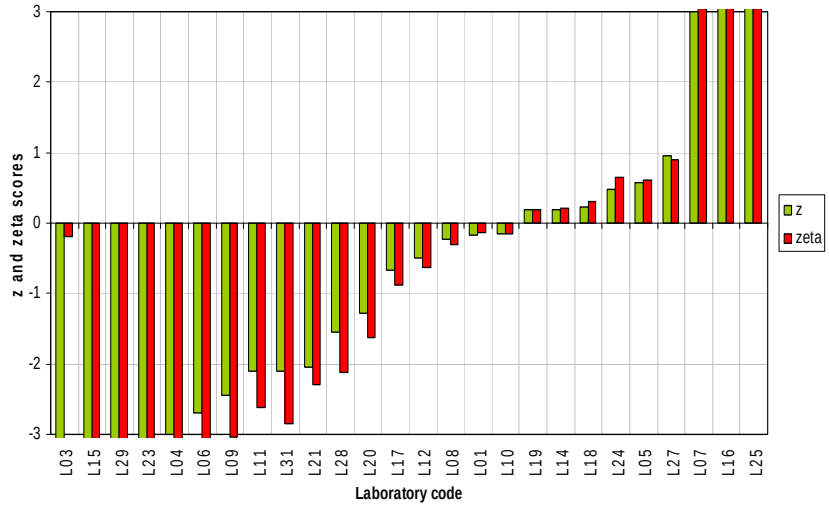

Figure 7c
Performance evaluation
$z = (x_{lab}-X_{ref})/ \hat{\sigma}$
$zeta = (x_{lab}-X_{ref})/ \sqrt{(u_{lab}^2+u_{ref}^2)}$

Table 2a: Total Cd, quantitative information reported by the participants plus the laboratory scorings provided by the organiser.
Total Cd content: 1,708 ± 0,056 mg kg^{-1}

Lab code	x_1	x_2	x_3	x_4	U_{lab}	k	Mean-calc	Technique	z	zeta
L01	1,69	1,69	1,75		0,41	2	1,71	ICP-MS	0,0	0,0
L02	1,649	1,748			0,141	2	1,699	ICP-MS	0,0	-0,1
L03	1,494	1,535	1,515		10	√3	1,515	ETAAS	-0,8	0,0
L04	1,86	1,72	1,83		0,27	2	1,80	ETAAS	0,4	0,7
L05	2,103	2,012	2,053		0,091	√3	2,056	ETAAS	1,4	4,6
L06	1,53	1,54	1,52		0	√3	1,53	ICP-OES	-0,7	-3,2
L07	1,70	1,72			0,23	2	1,71	ICP-OES	0,0	0,0
L08	1,62	1,61			0	√3	1,62	ICP-MS	-0,4	-1,7
L09	1,513	1,444	1,483		0,064	2	1,480	ETAAS	-0,9	-3,6
L10	1,084	1,187	0,975		0,227	2	1,082	ETAAS	-2,4	-5,0
L11	1,58	1,55	1,71		0,26	2	1,61	Zeeman ETAAS	-0,4	-0,7
L12	1,40	1,31	1,56		0,14	2	1,42	ICP-MS	-1,1	-3,2
L13	0,556	0,487	0,475		0,034	2	0,506	Flame AAS	-4,7	-20,9
L15	2,81	2,74	2,82		0,09	√3	2,79	ETAAS	4,2	14,3
L16	1,31	1,31	1,34		0,34	2	1,32	ICP-OES	-1,5	-2,2
L17	1,707	1,835			0,106	2	1,771	ICP-MS	0,2	0,8
L18	1,52				0,32	2	1,52		-0,7	-1,1
L19	1,69	1,52	1,72		0,28	2	1,64	ICP-MS	-0,3	-0,4
L20	1,60	1,68	1,67		0,16	√3	1,65	ICP-MS	-0,2	-0,5
L21	1,01	1,12	1,36		0,21	√3	1,16	ETAAS	-2,1	-4,1
L22	1,228	1,298	1,241		0,137	√3	1,256	ICP-MS	-1,8	-4,7
L23	1,083	1,134	1,117		0,109	2	1,111	AAS	-2,3	-7,7
L24	1,77	1,76			0	2	1,77	ETAAS	0,2	1,0
L25	2,676	2,498	2,680		0,524	2	2,618	ETAAS	3,6	3,4
L27	1,10	1,14			0,23	√3	1,12	ICP-OES	-2,3	-4,1
L28	1,57	1,52	1,52		0,23	2	1,54	Flame AAS	-0,7	-1,3
L29	1,45	1,5	1,4	1,43	0,18	2	1,45	Flame AAS	-1,0	-2,5
L31	1,424	1,540	1,473		0,12	2	1,479	ETAAS	-0,9	-2,8

All results expressed in mg kg^{-1}.

Table 2b: Total Pb, quantitative information reported by the participants plus the laboratory scorings provided by the organiser.
Total Pb content: 1,65 ± 0,19 mg kg^{-1}

Lab code	x_1	x_2	x_3	x_4	U_{lab}	k	Mean-calc	Technique	z	zeta
L01	1,44	1,42	1,42		0,44	2	1,43	ICP-MS	-0,9	-0,8
L02	0,952	0,943			0,012	2	0,948	ICP-MS	-2,8	-3,7
L03	0,637	0,617			10	√3	0,627	ETAAS	-4,1	-0,2
L04	0,76	0,62	0,72		0,10	2	0,70	ETAAS	-3,8	-4,8
L05	1,436	1,375	1,547		0,174	√3	1,453	ETAAS	-0,8	-0,9
L06	1,22	0,999	0,789		0	√3	1,00	ICP-OES	-2,6	-3,4
L07	1,79	1,85			0,22	2	1,82	ICP-OES	0,7	0,8
L08	1,31	1,38			0	√3	1,35	ICP-MS	-1,2	-1,6
L09	0,895	0,787	0,898		0,206	2	0,860	ETAAS	-3,2	-3,7
L10	0,471	0,458	0,512		0,12	2	0,480	ETAAS	-4,7	-5,9
L11	1,30	1,13	1,39		0,19	2	1,27	Zeeman ETAAS	-1,5	-1,8
L12	1,30	1,50	1,27		0,14	2	1,36	ICP-MS	-1,2	-1,4
L13	<0,095	<0,095	<0,095					Flame AAS		
L15	0,25	0,40	0,36		0,10	√3	0,337	ETAAS	-5,3	-6,6
L16	1,61	1,81	1,46		0,24	2	1,63	ICP-OES	-0,1	-0,1
L17	1,276	1,215			0,075	2	1,246	ICP-MS	-1,6	-2,1
L18	1,49				0,34	2	1,49		-0,6	-0,6
L19	1,41	1,88	1,55		0,31	2	1,61	ICP-MS	-0,1	-0,1
L20	1,06	1,02	1,02		0,10	√3	1,03	ICP-MS	-2,5	-3,1
L21	1,21	0,718	1,01		0,196	√3	0,979	ETAAS	-2,7	-3,0
L22	0,436	0,479	0,442		0,054	√3	0,452	ICP-MS	-4,8	-6,2
L23	0,425	0,430	0,413		0,041	2	0,423	AAS	-5,0	-6,4
L24	1,473	1,474			0	2	1,474	ETAAS	-0,7	-0,9
L25	2,700	2,670	2,795		0,680	2	2,722	ETAAS	4,3	2,8
L27	1,57	1,68			0,33	2	1,63	ETAAS	-0,1	-0,1
L28	1,22	1,32	1,20		0,38	2	1,25	ETAAS	-1,6	-1,5
L29	0,35	0,34	0,36	0,37	0,06	2	0,36	Flame AAS	-5,2	-6,7
L31	1,351	1,344	1,407		0,07	2	1,367	ETAAS	-1,1	-1,5

All results expressed in mg kg^{-1}.

Table 2c: Total As, quantitative information reported by the participants plus the laboratory scorings provided by the organiser.

Total As content: 1,18 ± 0,09 mg kg^{-1}

Lab code	x_1	x_2	x_3	x_4	U_{lab}	k	Mean-calc	Technique	z	zeta
L01	1,73	1,81	1,71		0,40	2	1,75	ICP-MS	3,1	2,7
L02	1,297	1,841			0,769	2	1,569	ICP-MS	2,1	1,0
L03	1,288	1,272	1,266		23	√3	1,275	HG-AAS	0,5	0,0
L04	0,90	0,82	0,86		0,13	2	0,86	HG-AAS	-1,7	-3,3
L05	1,60	1,65	1,74		0,18	√3	1,66	HG-AAS	2,6	3,9
L06	1,36	1,39	1,34		0,204	2	1,36	ETAAS	1,0	1,5
L07	1,15	1,13			0,07	2	1,14	HG-AAS	-0,2	-0,5
L08	1,50	1,46			0	√3	1,48	ICP-MS	1,6	4,3
L09	1,325	1,303	1,303		0,098	2	1,310	HG-AAS	0,7	1,5
L10	0,973	0,934	0,942		0,238	2	0.950	HG-AAS	-1,3	-1,7
L12	1,43	1,52	1,53		0,18	2	1,49	ICP-MS	1,7	2,7
L14	8,56	9,77			0	√3	9,17		43,7	114,1
L15	1,205	1,042	0,987		0,10	√3	1,078	HG-AAS	-0,6	-1,1
L17	1,305	1,329			0,132	2	1,317	ICP-MS	0,7	1,4
L18	1,28				0,24	2	1,28		0,5	0,7
L20	1,005	0,950	0,993		0,08	√3	0,983	HG-AAS	-1,1	-2,4
L21	0,937	1,02	1,21		0	√3	1,06	ETAAS	-0,7	-1,8
L22	0,927	0,985	0,939		0,119	√3	0,950	ICP-MS	-1,3	-2,3
L24	1,067	1,109			0	2	1,088	HG-AAS	-0,5	-1,3
L27	0,37	0,39			0,06	2	0,38	ETAAS	-4,4	-10,5
L28	1,43	1,53	1,44		0,44	2	1,47	ETAAS	1,6	1,2
L31	0,904	0,703	0,750	0,891	0,2	2	0,81	HG-AAS	-2,0	-3,0

All results expressed in mg kg^{-1}.

Table 2d: Extractable Cd, quantitative information reported by the participants plus the laboratory scorings provided by the organiser.
Extractable Cd content: 1,708 ± 0,056 mg kg^{-1}

Lab code	x_1	x_2	x_3	x_4	U_{lab}	k	Mean-calc	Technique	z	zeta
L01	1,58	1,58	1,62		0,38	2	1,59	ICP-MS	-0,4	-0,6
L03	1,769	1,688			10	√3	1,729	ETAAS	0,1	0,0
L04	1,72	1,64	1,69		0,25	2	1,68	ETAAS	-0,1	-0,2
L05	1,901	2,235	1,992		0,345	√3	2,043	ETAAS	1,3	1,6
L06	1,10	1,06	1,21		0	√3	1,12	ICP-OES	-2,3	-10,6
L07	1,84	1,68			0,24	2	1,76	ICP-OES	0,2	0,4
L08	1,62	1,69			0	√3	1,66	ICP-MS	-0,2	-1,0
L09	1,432	1,448	1,457		0,176	2	1,446	ETAAS	-1,0	-2,5
L10	1,965	1,875	2,069		0,372	2	1,969	ETAAS	1,0	1,3
L11	1,78				0,28	2	1,78	Zeeman ETAAS	0,3	0,5
L12	1,44	1,34			0,14	2	1,39	ICP-MS	-1,2	-3,6
L13	0,327	0,306	0,297		0,069	2	0,310	Zeeman ETAAS	-5,5	-21,5
L14	1,69	1,77			0,482	2	1,73	ETAAS	0,1	0,1
L15	2,37	2,58	2,48		0,09	√3	2,48	ETAAS	3,0	10,2
L16	1,48	1,45			0,37	2	1,47	ICP-OES	-0,9	-1,3
L17	1,764	1,757			0,106	2	1,761	ICP-MS	0,2	0,7
L18	1,40	1,33			0,099	2	1,37		-1,3	-4,6
L19	1,59	1,68	1,51		0,30	2	1,59	ICP-MS	-0,4	-0,7
L20	1,67	1,68	1,72		0,16	√3	1,69	ICP-MS	-0,1	-0,2
L21	0,741	0,985	1,28		0,18	√3	1,00	ETAAS	-2,8	-6,0
L23	1,077	1,054	1,112		0,098	2	1,081	AAS	-2,4	-8,5
L24	1,72	1,75			0	2	1,74	ETAAS	0,1	0,5
L25	2,159	1,965	2,211		0	√3	2,112	ETAAS	1,6	7,3
L27	1,21	1,16			0,24	2	1,19	ICP-OES	-2,0	-4,0
L28	1,49	1,51			0	√3	1,50	Flame AAS	-0,8	-3,8
L29	1,38	1,34	1,36	1,40	0,17	2	1,37	Flame AAS	-1,3	-3,3
L31	1,652	1,664	1,656		0,05	2	1,657	ETAAS	-0,2	-0,8

All results expressed in mg kg^{-1}.

Table 2e: Extractable Pb, quantitative information reported by the participants plus the laboratory scorings provided by the organiser.
Extractable Pb content: 1,29 ± 0,14 mg kg^{-1}

Lab code	x_1	x_2	x_3	x_4	U_{lab}	k	Mean-calc	Technique	z	zeta
L01	1,28	1,33	1,16		0,39	2	1,26	ICP-MS	-0,2	-0,1
L03	0,141	0,229			10	√3	0,185	ETAAS	-5,5	-0,2
L04	0,60	0,78	0,69		0,10	2	0,69	ETAAS	-3,0	-3,9
L05	1,487	1,294	1,436		0,199	√3	1,406	ETAAS	0,6	0,6
L06	0,713	0,815	0,725		0	√3	0,751	ICP-OES	-2,7	-3,7
L07	1,99	1,79			0,23	2	1,89	ICP-OES	3,0	3,2
L08	1,32	1,17			0	√3	1,25	ICP-MS	-0,2	-0,3
L09	0,845	0,764	0,793		0,134	2	0,801	ETAAS	-2,4	-3,1
L10	1,234	1,260	1,289		0,265	2	1,261	ETAAS	-0,1	-0,1
L11	0,87				0,13	2	0,87	Zeeman ETAAS	-2,1	-2,6
L12	1,20	1,18			0,12	2	1,19	ICP-MS	-0,5	-0,6
L13	<0,095	<0,095	<0,095					Flame AAS		
L14	1,41	1,25			0,226	2	1,33	ETAAS	0,2	0,2
L15	0,31	0,39	0,30		0,10	√3	0,333	ETAAS	-4,8	-6,1
L16	2,36	2,29			0,35	2	2,33	ICP-OES	5,2	4,6
L17	1,223	1,091			0,069	2	1,157	ICP-MS	-0,7	-0,9
L18	1,31	1,36			0,07	2	1,34		0,2	0,3
L19	1,20	1,35	1,44		0,32	2	1,33	ICP-MS	0,2	0,2
L20	1,12	1,02	0,96		0,10	√3	1,03	ICP-MS	-1,3	-1,6
L21	0,958	0,791	0,896		0,176	√3	0,882	ETAAS	-2,0	-2,3
L23	0,407	0,389	0,402		0,038	2	0,399	AAS	-4,5	-6,1
L24	1,41	1,36			0	2	1,39	ETAAS	0,5	0,7
L25	2,720	2,473	2,555		0	√3	2,583	ETAAS	6,5	8,9
L27	1,59	1,37			0,30	2	1,48	ETAAS	1,0	0,9
L28	0,98				0	√3	0,98	ETAAS	-1,6	-2,1
L29	0,36	0,39	0,37	0,38	0,07	2	0,38	Flame AAS	-4,6	-6,1
L31	0,881	0,861	0,871		0,03	2	0,871	Flame AAS	-2,1	-2,9

All results expressed in mg kg^{-1}.

Regarding the z and zeta scores, the results for the five measurands are summarised in Table3.

Table 3: Percentages of laboratories scoring satisfactory, questionable and unsatisfactory.

	Total Cd		Total Pb		Total As		Extractable Cd		Extractable Pb	
	Numb. Labs	%	Numb. Labs	%	Numb. Labs	%	Numb. Labs	%	Numb. Labs	%
z										
Satisf.	**21**	**75**	**14**	**52**	**17**	**77**	**22**	**81**	**14**	**54**
Quest.	**4**	**14**	**4**	**15**	**2**	**9**	**4**	**15**	**6**	**23**
Unsat.	**3**	**11**	**9**	**33**	**3**	**14**	**1**	**4**	**6**	**23**
zeta										
Satis.	**13**	**46**	**14**	**52**	**12**	**54**	**15**	**55**	**13**	**50**
Quest.	**3**	**11**	**3**	**11**	**5**	**23**	**1**	**4**	**4**	**15**
Unsat.	**12**	**43**	**10**	**37**	**5**	**23**	**11**	**41**	**9**	**35**

The results in this proficiency test are not as satisfactory as in the four previous exercises organised by the CRL-HM for the network of NRL's[16], probably due to an inherent difficulty of the matrix. Improvement is certainly needed in the determination of total and extractable Pb in which hardly half of the participants obtained a satisfactory z-score.

As expected, the situation does not look better when taking into consideration the zeta-scores. Only half of the participants obtained a satisfactory zeta-score, and not only for Pb determinations but also for all the measurands included in this exercise. Between 35 and 40 % of the participants obtained an unsatisfactory zeta-score for all the measurands with the exception of total As, for which 23 % of the laboratories had a |zeta| > 3. This means that participants have had problems not only with the accuracy of their method but also in making a sound estimation of the uncertainty characterising their measurements.

Evaluating the information provided by the participants in the questionnaire (Annex 5) it was observed that most of the laboratories have optimised their method for the determination of total Cd, Pb and As using certified reference materials of mainly organic composition. Table 4 summarises the CRM's used for the validation of the methods as reported by the participants. Organic matrices are easier to mineralise than matrices of mineral origin, which may easily result in an overestimation of the recovery and thus in an underestimation of the analyte concentration when the same method is applied to a mineral matrix. This hypothesis would explain why according to the Youden plot the values reported for total Pb were more biased that those reported for extractable Pb. The concentration of extractable Pb is method-dependent and have been obtained by all the laboratories using the same method. When developing and validating a method to analyse heavy metals in mineral feed, it would be more appropriate to use a sediment CRM if no mineral feed CRM is available on the market.

However, it must be noticed that in this exercise also laboratories with a mandate for analysis of heavy metals in food only, have taken part. Those laboratories have participated on a voluntary basis in this interlaboratory comparison and do not necessarily have experience with this type of matrix which has no similarity with any food commodity.

Table 4: Certified Reference Materials used by the laboratories taking part in this exercise for the validation of the methods used for the analysis and in some cases for calibration purposes, as reported by the participants.

Lab code	Do you use CRMs?	Is the CRM used for validation?	Is the CRM used for calibration?	Which CRM do you use?
L01	Yes	Yes	No	NRC Tort 2 (lobster hepatopancreas), LGC 7162 (?)
L02	Yes	Yes	No	CE 278 (mussel tissue), BCR 185 (bovine liver), IAEA-359 (cabbage)
L03	Yes	Yes	Yes	NIST Wheat Flour and NIST Wheat Gluten
L04	Yes	Yes	No	BCR-191 (wheat flour)
L06	Yes	Yes	Yes	NIST 1568a (rice flour)
L07	Yes	Yes	No	BCR 279 (sea lettuce), BCR 627 (tuna fish), ARC/CL total diet reference material (HDP)
L08	Yes	Yes	No	Corn bran
L09	Yes	Yes	No	DC 73348 (?), NIST 1547 (peach leaves)
L10	Yes	Yes	No	
L11	Yes	Yes	No	
L12	Yes	Yes	No	Lucerne-P- ALFALFA
L15	Yes	Yes	Yes	Brown bread powder
L16	Yes	Yes		IAEA V10 hay powder
L17	Yes	Yes	No	Internal reference material from interlaboratory comparison organised by our own institute
L18	Yes	Yes	No	
L19	Yes	Yes	No	IAEA 407 (fish homogenate)
L20	Yes	Yes	No	
L22	Yes	Yes	Yes	ICP-MS, AAS
L23	Yes	Yes	Yes	Merck 1.197/6.0500, Merck 1,9/77,0500
L24	Yes	Yes	No	
L25	Yes	Yes	No	BCR-279 (Sea lettuce) & SRM 1573a (tomato leaves)
L27	Yes	Yes	No	IMEP-103 and 104
L28	Yes	Yes	Yes	CRM

Table 5 describes in detail the method used by IRMM for the determination of total Cd, Pb and As in mineral feed. This method was used to obtain the reference values for the mentioned measurands. Table 5 also contains the method applied by Bayer Antwerpen for the determination of total As in the test material to perform the homogeneity and stability studies. The results obtained by Bayer Antwerpen are in agreement with the reference value provided for total As by SCK-CEN. The method used by SCK is neutron activation analysis, which is not a technique commonly available neither in the NRLs nor in routine control laboratories. The methods summarised in Table 5 could be used as starting point for laboratories having problems with the determination of total Cd, Pb and As and that want to improve their performance.

Table 5: Summary of methods that have been used for the determination of total Cd and Pb by IRMM, and of total As by Bayer Antwerpen, on the test material used in this exercise.

Method	**Procedure**
Method used by IRMM to determine total Cd and Pb.	About 0.2 g of feed sample were weighed into a microwave Teflon boat. 4 mL of HNO_3 (65%), 2 mL of HCl (36%) and 1 mL HF (49%) were added to the microwave vessel and left for 2 h for the pre-digestion of the samples. The digestion in the microwave was done applying the following programme: **1)** 2 min at 250 W, **2)** 2 min at 0 W, **3)** 6 min at 250 W, **4)** 2 min at 0 W, **5)** 5 min at 650 W, **6)** 2 min at 0 W, **7)** 5 min at 650 W, **8)** 20 min at 0 W. After finishing the microwave decomposition, the Teflon vessels were put into a heating block and the sample digests are evaporated almost to dryness. The digest and reference materials dilutions were made with 2% HNO_3. The procedural blank samples followed exactly the same digestion procedure as the feed samples.
Method used by Bayer Antwerpen to determine total As	Sample preparation: Extraction with reflux boiling with concentrated HCl 1. Weigh 600 mg of the prepared test sample to the nearest mg into a 100 mL flask. 2. Add 25 mL concentrated HCl (p.a. Baker) and 25 mL milli-Q-water and place a reflux cooler on the flask. 3. Put the flask and cooler on a hot plate and let the solution boil for a total time of one hour. 4. After 20 minutes, add dropwise 10 mL H_2O_2 (w/w) (p.a. Baker) via the dosing capillary. 5. Wait 20 minutes before flushing the capillary with 10 mL milli-Q-water. 6. Leave the flask and the cooler on the hot plate for another 20 minutes. 7. Remove the flask and the cooler from the hot plate, allow to cool down and rinse the cooler with milli-Q-water. 8. Add 2 ml of KI solution and dilute to the mark with milli-Q-water. 9. Leave the solution to stand for another 50 minutes before starting the measurements. Measurement: HG-AAS on Varian SpectrAA-300 with VGA-76 hydride generator Wavelength: 193,7 nm Dilution: the extraction solution (8) was measured undiluted Calibration: 5, 10, 15 µg/L Quality Control: samples spiked with 5 µg/L As

Additional information was gathered from the questionnaire completed by the participants. Eleven laboratories have corrected their results for recovery and eighteen did not. Of those than did eight calculated the recovery using a reference material and three adding a known amount of the same analyte to be measured. Of those that did not correct the results for recovery one said that it was because they do not have an appropriate reference material, one indicated that they did not correct for recovery because the recovery for the determination of all elements was in the range covered by the uncertainty. Several participants mentioned that no correction was made because the recovery was carefully evaluated during the method validation and it turn out to be close to 100 %. Five laboratories answered that they do not correct for recovery in this type of analysis. One laboratory indicated that the results where checked from different solutions, with different techniques (ICP-OES and flame AAS) and with the use of a reference material.

When asked about the level of confidence reflected by the reported coverage factor (k), twenty one reported a level of 95 %, one of 90 % and seven did not reported any figure. For uncertainty estimates, various combinations of two or more options (question 3 of the questionnaire shown in Annex 5) were given. Eighteen laboratories use the uncertainty as calculated during in-house validation of the method, eleven laboratories use the uncertainty obtained by measuring replicates (i.e. precision). Eight laboratories applied the ISO-GUM. Three participants used the known uncertainty of the standard method used for the analysis. Three laboratories made use of intercomparison data and one used the expert guesstimate. Eighteen laboratories provide an uncertainty statement to their customers and eleven do not.

Twenty-six laboratories corrected their results for the water content and three did not. Laboratory L23 indicated that they did not correct for the moisture content because they have pre-dried the sample before the analyses. Also L3 reported to have dried the samples before carrying out the analysis of extractable Pb. The way in which the moisture content of the test material was to be calculated was described in the detail in the accompanying letter, but in that letter it was not specified that the aliquot used to determine the moisture content should not be used in further analyses. This fact could have caused some confusion among some participants.

Four laboratories reported to have introduced some modification to the prescribed protocol for the partial extraction of Cd and Pb: L03 pre-dried the sample, as previously mentioned, L07 took 1 g of sample instead of 2 g, L09 weighed 2 g of sample in a 250 mL flask, then added 143 mL extraction solution (5% HNO_3), and after the heating filled up the 250 mL volumetric flask with water, L29 used 4 g of sample and 30 mL of HNO_3.

All participants but one use total matrix digestion in routine analysis, the remaining laboratory performs both total and partial extraction (according to legislation).

Fourteen laboratories analysed the test material following an official method. The information reported by the remaining laboratories about their method of analysis is summarised in Annex 6.

Twenty-five participants carry out this type of analysis on a routine basis, four do not and one did not answer this question.

Twenty-seven laboratories have a quality system in place, one does not and one did not answer the question. One out of the twenty-seven has the quality system based on both ISO 17025 and ISO 9000 series. The remaining twenty-six have their quality system based on ISO 17025. Five laboratories are not accredited for the type of analysis that they apply on a routine basis.

Twenty-six laboratories participate regularly in ILC's for this type of analysis.

Twenty-three participants use a reference material for this types of analyses, out of which six use it not only for the validation of the method but also for calibration purposes.

No cluster of results was observed as function of the technique used to perform the analysis for any of the measurands included in the exercise. Figure 8 shows the distribution of the z-scores for total Pb as function of the technique used after applying multivariant analysis. Further information obtained from the multivariant analysis will be collected in another report.

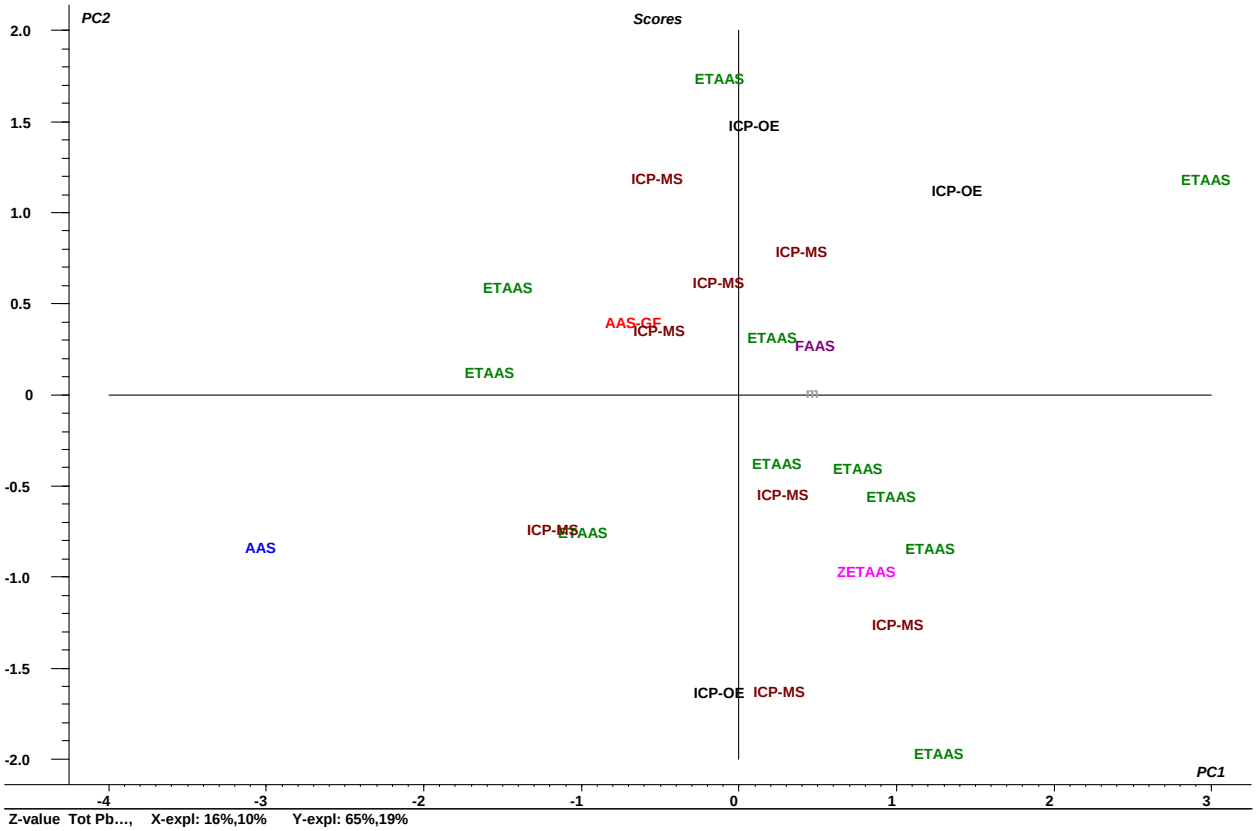

Figure 8: Distribution of z-scores for total Pb in function of the technique used as derived from multivariant analysis (projection to latent structures (PLS) relating the Pb z-score to the answers provided by the laboratories from the questionnaire).

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9 Conclusions

The first conclusion that can be withdrawn from this exercise is that the selection of the reference material used to evaluate the recovery and/or to validate the method of analysis, is of paramount importance. As shown as outcome of this exercise, using reference materials that do not match the type of matrix of the test material itroduces a significant underestimation of some of the measurands, such as in this case of total Pb.

The second conclusion that can be derived from this exercise is that the concentration of total and extractable Cd (according to Directive 2002/32/EC) can be considered identical when analysing mineral feed. The same conclusion was extracted from IMEP-103 when analysing feed with a high content of organic matter[17]. Nevertheless, a wider variety of feed matrices should be checked before extracting general conclusions. This conclusion does not apply when comparing total and extractable Pb content in mineral feed, in which the concentration of total Pb is higher that the concentration of extractable Pb, as deduced from the reference values and as confirmed by the results of some participants in the exercise.

There seems to be some confusion among the participants on whether results are to be corrected for recovery or not. According to Commission Regulation (EC) No 333/2007: *"If an extraction step is applied in the analytical method, the analytical result shall be corrected for recovery. In this case the level of recovery must be reported. In case no extraction step is applied in the analytical method (e.g. in case of metals), the result may be reported uncorrected for recovery if evidence is provided by ideally making use of suitable certified reference material that the certified concentration allowing for the measurement uncertainty is achieved (i.e. high accuracy of the measurement). In case the result is reported uncorrected for recovery this shall be mentioned".* This means that during the validation of a method the laboratory must perform studies to evaluate the accuracy of the method, ideally using CRMs when available. When the result is not biased and it falls in the concentration range provided by the CRM (taking into consideration the uncertainty of the certified value), or when there is an overlap of the concentration range obtained by the laboratory (taking into consideration the uncertainty of the measurement as evaluated by the lab), then there is no need to correct for recovery and so it must be reported to the customer, as indicated in the legislation. Otherwise, the results are to be corrected for recovery. Analysts must keep in mind that extractions with organic solvents are not the only steps in an analytical procedure that could introduce a low recovery. Precipitation, volatilisation, incomplete digestion and adsorption are, among others, possible sources of biased results.

10 Acknowledgements

C. Contreras and A. Lamberty from the Reference Materials Unit are acknowledged for their support in the processing of the test material. The Central Laboratory of Bayer Antwerpen is acknowledged for performing the measurements for the homogeneity and stability studies. The authors thank the Studiecentrum voor Kernenergie (SCK-CEN) and in particular P. Vermarke, for providing the reference value for total As. P. Robouch is acknowledged for the support in the construction and interpretation of the Youden plots.

The NRLs participating in this exercise, listed below are kindly acknowledged.

Organisation	Country
AGES Zentrum fur Analytik und Mikrobiologie	Austria
AGES Competece Centre of Elements	Austria
Veterinary and Agrochemical Research Centre CODA-CERVA	Belgium
Central Laboratory of Veterinary Control and Ecology	Bulgaria
SVI Olomuc	Czech Republic
Central Institute for Supervising and Testing in Agriculture	Czech Republic
Danish Plant Directorate	Denmark
Agricultural Research Centre	Estonia
Veterinary and Food Laboratory	Estonia
Evira	Finland
Service Commun des Laboratoires	France
Federal Office of Consumer Protection and Food Safety	Germany
General Chemical State Laboratory	Greece
Regional Center of Plant Protection and Quality Control of Magnisia	Greece
Central Agricultural Office, Food and Feed Safety Directorate	Hungary
Cork Public Analyst Laboratory	Ireland
Istituto Zooprofilattico Sperimentale	Italy
National Diagnostic Centre	Latvia
National Food and Veterinary Risk Assessment Institute	Lithuania
Public Health Labortory	Malta
National Veterinary Research Institute	Poland
Laboratorio Nacional de Investigaçao Veterinaria	Portugal
Hygiene and Veterinary Public Health Institute	Romania
State Veterinary and Food Institute	Slovakia
National Veterinary Institute	Slovenia
Laboratorio Arbitral Agroalimentario	Spain
National Food Administration	Sweden
National Veterinary Institute	Sweden
Food and Consumer Product Safety Authority	The Netherlands

Countries not appearing on the above list did not reported results to this ILC.

11 References

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[16] http://irmm.jrc.ec.europa.eu/html/CRLs/crl_heavy_metals/index.htm
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Annexes

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Annex 1: Invitation letter to laboratories

EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

Institute for reference materials and measurements
Community reference laboratory for heavy metals in feed and food

CRL Heavy Metals in Feed and Food

Geel, 3 October 2008
D04-IM/BCa/ive(2008)D/25121

«Title» «M_1st_name» «last_name»
«Institute»
«Department»
«Address»
«DHL_delivery_address»
«ZIP» «City»
«COUNTRY»

Inter-laboratory comparison for CRL Heavy Metals in Feed and Food

Dear «Title» «last_name»,

On behalf of the CRL Heavy Metals in Feed and Food, I would like to invite you to participate in the Proficiency Test [IMEP-105] for the determination of **total** Cd, Pb and As and **extractable amounts** of Cd and Pb in feed following Directive 2002/32/EC of the European Parliament and of the Council on undesirable substances in animal feed.

I would like to remind you that – according to Regulation (EC) No 882/2004 - you have the duty as NRL to participate in PTs organised by the CRL if you hold a mandate for the type of matrix investigated.

Please register electronically for this inter-laboratory comparison using the following link:
http://www.irmm.jrc.be/ilc/ilcRegistration.do?selComparison=180

Your participation is free of charge.

Once you have submitted your registration electronically, please follow the procedure indicated: a) print your registration form; b) sign it; and c) fax it to us. **Your fax is the confirmation of your participation.**

Retieseweg 111, B-2440 Geel - Belgium. Telephone: (32-14) 571 211. http://irmm.jrc.ec.europa.eu
Telephone: direct line (32-14) 571 299. Fax: (32-14) 571 865.

E-mail: jrc-irmm-crl-heavy-metals@ec.europa.eu

The **deadline for registration is 15 October 2008**. Samples will be sent to participants during the second half of October. The deadline for submission of results is 30 November 2008.

I am the project leader for this inter-laboratory comparison. In case of questions/doubts, do not hesitate to contact me.

Yours sincerely,

Dr. Maria Beatriz la Calle
Deputy-Operating Manger CRL-HM

Cc: Philip Taylor

2

Annex 2: Results of the homogeneity and stability studies

1a. Homogeneity data for total As in mineral feed

According to the IUPAC International Harmonised Protocol[8]

	As (mg kg^{-1})	
Bottle ID	**Replicate 1**	**Replicate 2**
13	1,03	1,13
53	1,01	1,17
77	1,16	1,15
120	1,09	1,12
145	1,13	1,11
170	1,13	1,11
208	1,11	1,13
214	1,13	1,1
264	1,15	1,18
293	1,15	1,11
Mean, n	**1.12**	**20**
Target RSD %	16	
S_{an}^2	0.00206	
S_{sam}^2	-0,000352222	
σ_{all}^2	0,002782774	
Critical	0,007312214	
S_{sam}^2<critical?	ACCEPT	

According to ISO 13528[3]

0.3σ	0,052752
s_x	0,026034166
s_w	0,045387223
s_s	#NUM!
$s_s \leq 0.3\ \sigma$	ACCEPT

1.b Stability data for total As in mineral feed

As computed by SOFT CRM

TEMPERATURE = 18°C

		Time in Weeks			
samples		0	3	5	8
	1	1,11	1,15	1,05	1,22
	2	1,2	1,18	1,14	1,23

CALCULATION OF u_{sts} for given Xshelf
Given Xshelf = 6 Weeks
U_b =0,007

u_{sts} = 0,044
u_{sts}[%] = 3,8%

Slope = 0,006
SE Slope = 0,008
Intercept = 1,135
SE Intercept = 0,037
Correlation Coefficient =0,101

Slope of the linear regression significantly <> 0 (95%) :No
Slope of the linear regression significantly <> 0 (99%) :No

2a. Homogeneity data for extractable Cd in mineral feed

According to the IUPAC International Harmonised Protocol[8]

	Cd (mg kg^{-1})	
Bottle ID	**Replicate 1**	**Replicate 2**
13	1,73	1,74
53	1,69	1,73
77	1,78	1,78
120	1,77	1,7
145	1,77	1,76
170	1,76	1,71
208	1,76	1,81
214	1,69	1,67
264	1,66	1,69
293	1,82	1,75
Mean, n	**1,7385**	**20**
Target RSD %	15	
S_{an}^2	0,000895	
S_{sam}^2	0,001225	
σ_{all}^2	0,005877959	
Critical	0,011954513	
S_{sam}^2<critical?	ACCEPT	

According to ISO 13528[3]

0.3σ	0,07666785
$\mathbf{s_x}$	0,04089621
$\mathbf{s_w}$	0,029916551
$\mathbf{s_s}$	0,035
$\mathbf{s_s \leq 0.3\ \sigma}$	ACCEPT

2.b Stability data for extractable Cd in mineral feed

As computed by SOFT CRM

TEMPERATURE = 18°C

		Time in Weeks			
samples		0	3	5	8
	1	1,66	1,75	1,78	1,72
	2	1,76	1,77	1,8	1,82

CALCULATION OF u_{sts} for given Xshelf
Given Xshelf = 6 Weeks
U_b =0,006

u_{sts} = 0,036
u_{sts}[%] = 2,1%

Slope = 0,008
SE Slope = 0,006
Intercept = 1,726
SE Intercept = 0,028
Correlation Coefficient =0,247

Slope of the linear regression significantly <> 0 (95%) :No
Slope of the linear regression significantly <> 0 (99%) :No

3a. Homogeneity data for extractable Pb in mineral feed

According to the IUPAC International Harmonised Protocol[8]

	Pb (mg kg^{-1})	
Bottle ID	**Replicate 1**	**Replicate 2**
13	1,27	1,35
53	1,44	1,36
77	1,08	1,13
120	1,19	1,09
145	1,17	1,07
170	1,18	1,39
208	1,31	1,16
214	1,43	1,55
264	1,2	1,12
293	1,07	1,05
Mean, n	**1,2305**	**20**
Target RSD %	15.5	
S_{an}^2	0,006155	
S_{sam}^2	0,016430556	
σ_{all}^2	0,003273928	
Critical	0,012371535	
S_{sam}^2<critical?	NOT ACCEPT	

According to ISO 13528[3]

0.3σ	0,05721825
s_x	0,139671241
s_w	0,078453808
s_s	0,128181729
$s_s \leq 0.3\ \sigma$	NOT ACCEPT

3.b Stability data for extractable Pb in mineral feed

As computed by SOFT CRM

TEMPERATURE = 18°C

	Time in Weeks			
samples	0	3	5	8
1	1,04	1,46	1,26	1,39
2	1,12	1,19	1,12	1,42

CALCULATION OF u_{sts} for given Xshelf
Given Xshelf = 6 Weeks
U_b =0,019

u_{sts} = 0,115
u_{sts}[%] = 9,2%

Slope = 0,034
SE Slope = 0,015
Intercept = 1,113
SE Intercept = 0,075
Correlation Coefficient =0,458

Slope of the linear regression significantly <> 0 (95%) :No
Slope of the linear regression significantly <> 0 (99%) :No

Annex 3: Letter accompanying the sample

EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

Institute for reference materials and measurements
Community reference laboratory for heavy metals in feed and food

CRL Heavy Metals in Feed and Food

Geel, 20 October 2008
D04-IM(2008)BdlC/ive/D/26282

«TITLE» «FIRSTNAME» «SURNAME»
«ORGANISATION»
«DEPARTMENT»
«ADDRESS»
«ADDRESS2»
«ADDRESS3»
«ADDRESS4»
«ZIP» «TOWN»
«COUNTRY»

Participation to IMEP-105, a proficiency test exercise for the determination of total Cd, Pb and As and extractable amounts of Cd and Pb in mineral feed

Dear «TITLE» «SURNAME»,

Thank you for participating in the IMEP-105 intercomparison for the determination of **total** Cd, Pb and As and **extractable amounts** of Cd and Pb in mineral feed. This exercise takes place in the frame of the CRL Heavy Metals in Feed and Food.

This parcel contains:
a) One glass bottle containing approximately 20 g of the test material
b) A "Confirmation of Receipt" form
c) This accompanying letter

Please check whether the bottle containing the test material remained undamaged during transport. Then fax (at +32-14-571865) or send the "Confirmation of receipt" form back. You should store the samples in a dark and cold place (not more than 18 °C) until analysis.

The measurands are: **total** Cd, Pb and As and **extractable** amounts of Cd and Pb according to Directive 2002/32/EC of the European Parliament and of the Council on undesirable substances in animal feed, in a mineral feed matrix.

As agreed upon during the workshop held in September, the determination of the **extractable** amounts of Cd and Pb shall be carried out by strictly applying the following procedure:

«PARTKEY»

Retieseweg 111, B-2440 Geel - Belgium. Telephone: (32-14) 571 211. http://irmm.jrc.ec.europa.eu
Telephone: direct line (32-14) 571 252. Fax: (32-14) 571 865.

E-mail: jrc-irmm-crl-heavy-metals@ec.europa.eu

Protocol for the partial extraction of Cd and Pb in mineral feed

(IMEP-105)

1. *Weigh about 2 g of the prepared test sample to the nearest 1 mg into a 250 mL beaker.*
2. *Add 85 mL of a 5 % (w/w) HNO_3 solution (see note for the preparation of the HNO_3 solution).*
3. *Cover the beaker with a watch-glass and boil for 30 min on a hot plate (make sure that the plate warms up homogeneously all over the surface).*
4. *Allow to cool. Decant the liquid into a 100 mL volumetric flask, rinsing the beaker and the watch-glass several times with 5 % (w/w) HNO_3.*
5. *Dilute to the mark with 5 % (w/w) HNO_3.*
6. *After homogenising, filter through a fry folded filter paper into a dry container. Use the first portion of the filtrate to rinse the glassware and discard that part. If the determination is not carried out immediately, the container with filtrate shall be stoppered.*
7. *Carry out a blank test at the same time as the extraction, with only the reagents and follow the same procedure as for the samples.*

To construct the calibration curve dilute the standards in 5 % (w/w) HNO_3.

NOTE: To prepare 1 kg stock of 5 % (w/w) HNO_3 (density ~ 1.0257 kg/l): mix 77 g of 65 % (w/w) HNO_3 with 923 g water. Use a balance of two digits for the weighing.

For the determination of the **total** content of Cd, Pb and As the procedure that you use should resemble as closely as possible the one that you use in routine sample analysis.

Please perform two or three independent measurements per measurand. Correct the measurement results for recovery, and report the corrected values, plus their mean on the reporting website. The results should be reported in the same form (e.g., number of significant figures) as those normally reported to the customer.

The results are to be reported referring to dry mass and thus corrected for humidity. To calculate the water content in the test material, please apply the following procedure:

Weigh 2 g of test material and dry it at 103 ± 2 °C for 4 hours in triplicate

You can find the reporting website at https://irmm.jrc.ec.europa.eu/ilc/ilcReporting.do
To access this webpage you need a personal password key, which is: «PARTKEY». The system will guide you through the reporting procedure. Please enter for each parameter the two or three measurement results plus the technique you used, but do not report the uncertainty for each individual measurement. In addition, please report the mean of the results with technique and with uncertainty information in the allocated space for "measurement 4". After entering all results, please also complete the relating questionnaire. Do not forget to save, submit and confirm always when required.

Directly after submitting your results and the questionnaire information online, you will be prompted to print the completed report form. Please do so, sign the paper version and return it to IRMM by fax (at +32-14-571-865) or by e-mail. Check your results carefully for any errors before submission, since this is your definitive confirmation.

The deadline for submission of results is 30/11/2008.

Please keep in mind that collusion is contrary to professional scientific conduct and serves only to nullify the benefits of proficiency tests to customers, accreditation bodies and analysts alike.

Your participation in this project is greatly appreciated. If you have any remaining questions, please contact me by e-mail:
JRC-IRMM-CRL-HEAVY-METALS@ec.europa.eu

With kind regards

Dr. M.B. de la Calle
IMEP-105 Co-ordinator

Enclosures: 1) one glass bottle containing the test material; 2) confirmation of receipt form; 3) Accompanying letter.

Cc: P. Taylor

Annex 4: Sample receipt confirmation form

EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

Institute for reference materials and measurements
Community reference laboratory for heavy metals in feed and food

CRL Heavy Metals in Feed and Food

Annex to D04-IM(2008)BCa/ive/D/26282

«TITLE» «FIRSTNAME» «SURNAME»
«ORGANISATION»
«DEPARTMENT»
«ADDRESS»
«ADDRESS2»
«ADDRESS3»
«ZIP» «TOWN»
«COUNTRY»

CRL-HM-05 / IMEP-105

total Cd, Pb and As and extractable amounts of Cd and Pb in mineral feed

Confirmation of receipt of the samples

Please return this form at your earliest convenience.
This confirms that the sample package arrived.
In case the package is damaged,
please state this on the form and contact us immediately.

ANY REMARKS ……
……

Date of package arrival ……

Signature ……

Please return this form to:
Dr Beatriz de la Calle

IMEP-105 Coordinator
EC-JRC-IRMM
Retieseweg 111
B-2440 GEEL, Belgium

Fax : +32-14-571865
e-mail : JRC-IRMM-CRL-HEAVY-METALS@ec.europa.eu

Retieseweg 111, B-2440 Geel - Belgium. Telephone: (32-14) 571 211. http://irmm.jrc.ec.europa.eu
Telephone: direct line (32-14) 571 252. Fax: (32-14) 571 865.

E-mail: jrc-irmm-crl-heavy-metals@ec.europa.eu

IMEP

Annex 5: Questionnaire

ilc questionnaire

Comparison for IMEP-105

Please fill in this questionnarie

Submission Form

1. **Did you apply a recovery factor to correct your measurement results?**

- ○ no
- ○ yes

1.1. If Yes, what are the recovery factors (R, in %) you used:

1.1.1. for Cd (in %)

1.1.2. for Pb (in %)

1.1.3. for As (in %)

1.2. If Yes, did you determine R by:

- ☐ a) adding a known amount of the same analyte to be measured (spiking)
- ☐ b) using a reference material
- ☐ c) other

1.2.1. If other, please specify.

1.3. If No, please state why:

2. **What is the level of confidence reflected by the coverage (k) factors stated above? (in %)**

3. **What is the basis of your uncertainty estimate (multiple answers are possible)**

- ☐ a) uncertainty budget according to ISO-GUM
- ☐ b) known uncertainty of the standard method
- ☐ c) uncertainty of the method as determined during in-house validation
- ☐ d) measurement of replicates (i.e. precision)
- ☐ e) expert guesstimate
- ☐ f) use of intercomparison data

☐ g) other

3.1. If other, please specify.

4. **Do you usually provide an uncertainty statement to your custumers for this type of analysis?**

○ no
○ yes

5. **Did you correct for the water content of the sample?**

○ No
○ Yes

5.1. If Yes, what is the water content (in % of the sample mass)?

5.2. If No, what was the reason not to do this?

6. **Did you modify the prescribed protocol for the partial digestion?**

○ no
○ yes

6.1. If yes, please specify the modifications introduced.

7. **Did you analyse the sample according to an official method?**

○ no
○ yes

7.1. If No, please describe (in max. 150 characters for each reply) your:

7.1.1. sample pre-treatment

7.1.2. digestion step

7.1.3. extraction / separation step

7.1.4. instrument calibration step

7.2. If Yes, which:

8. **Does your laboratory carry out this type of analysis (as regards the parameters, matrix and methods) on a routine basis?**

- ○ no
- ○ yes

8.1. If Yes, please estimate the number of samples (As, Cd, Pb measurements together):

- ☐ a) 0-50 samples per year
- ☐ b) 50-250 samples per year
- ☐ c) 250-1000 samples per year
- ☐ d) more than 1000 samples per year

9. **Does your laboratory have a quality system in place?**

- ○ no
- ○ yes

9.1. If Yes, which:

- ☐ a) ISO 17025
- ☐ b) ISO 9000 series
- ☐ c) Other

9.1.1. If other, please specify.

10. **Which type of sample treatment do you routinely use for such samples?**

- ☐ Partial digestion (according to the legislation)
- ☐ Total digestion

11. **Is your laboratory accredited for the sample treatment that you specify in question 10?**

- ○ No
- ○ Yes

12. **Does your laboratory take part in an interlaboratory comparison for this type of analysis on a regular basis?**

- ○ no
- ○ yes

12.1. If yes, which one(s):

13. **Does your laboratory use a reference material for this type of analysis?**

- ○ no
- ○ yes

13.1. If YES, is the material used for the validation of procedures?

- ○ no
- ○ yes

13.2. If YES, is the material used for calibration of instruments?

- ○ no
- ○ yes

13.3. If yes, which one(s)

14. **Do you have any comments? Please let us know: ...**

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Annex 6: Experimental details

Lab code	Official Standard method?	Which official standard method?	Sample pre-treatment	Digestion step	Extraction/separation step	Instrumental calibration
L01	No		0,5 g sample	microwave digested with 4 mL nitric acid, 2 mL hydrogen peroxide and 2 mL water. Made up to 200 mL		7 point calibration. 0-20 pbb standards
L02	No		homogeneise	microwave digestion	n.a.	calibration by using certified standard solutions
L03	Yes	EN 15550:2007				
L04	No			nitric acid, microwave digestion, 220 ºC, 40 min		External standards
L05	Yes	AOAC 999.11				
L06	Yes	CEN/TS 15621:2007; EN 14332:2004				
L07	No			Pb/Cd: Ashing at 470 C. As: Ashing with ashing aid at 595 ºC	Pb/Cd 37 % HCl evaporation and then extraction with 2% HCl. As: Extraction with 6 M HCl	External calibration with correction for spike recovery
L08	No	ICP.MS calibration standards	None	microwave ac. Nitric		Yes
L09	No			Open digestion with conc. HNO_3 for Cd and Pb, and open digestion with conc. HNO_3/$HCLO_4$ for As		External calibration for all methods
L10	No		Drying in oven	Pb and Cd ashing in mufle at 450 ºC, dissolved in HCl; As ashing with magnesium nitrate hexahydrate solution nitrate magnesium		Calibration curve for Pb (10-60 µg/L); Cd (1-10 µg/L); As (3-25 µg/L)
L11	No		None	Digestion with H_2O_2 (30%) and HNO_3 conc. By microwave high pressure		Add. Method; std solution Cd: 2 ppb, Pb: 50 ppb

L12	Yes	AOAC				
L13	Yes	EN 14082				
L14	Yes					
L15	No		Drying 103 ºC 4 hours	5% (W/W) nitric acid	Decantation	Standard
L16	No			Bomb digestion, 4 mL conc nitric acid 0,4 g sample	After digestion dilution to 10 mL with water	External calibration, using commercial stock standards
L17	No			2 g of sample + 20 mL of nitric acid (10 mol/L)		ICP-MS measurements with octopole reaction system and internal standard calibration
L18	Yes					
L19	No		5 mL HNO_3 + 1 mL HCl + 1 mL HF	Microwave digestion 180 ºC and 600 W for 35 min		Standard solutions of Cd and Pb
L20	Yes					
L21	Yes	ASU L 00,00-19/3				
L22	Yes					
L23	No		add 2 mL 65% HNO_3	microwave	dilution	4 step
L24	Yes					
L25	Yes	AOAC Official method 999,10				
L27	No		No	Digestion with concentrate nitric acid (5 mL for 0.5 g)		5 levels
L28	Yes	NMKL 161/1998				
L29	Yes	SR EN 14082:2003				
L31	No		None	0,5 g sample with 5 mL HNO_3 microwave digestion at 200 ºC for 15 minutes	None	Yes

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Abstract
The Institute for Reference Materials and Measurements (IRMM) of the Joint Research Centre, a Directorate-General of the European Commission, operates the Community Reference Laboratory for Heavy Metals in Feed and Food (CRL-HM). One of its core tasks is to organise interlaboratory comparisons (ILCs) among appointed National Reference Laboratories (NRLs). This report presents the results of the fifth ILC of the CRL-HM which focused on the determination of total Cd, Pb and As and extractable Cd and Pb in mineral feed according to Directive 2002/32/EC of the European Parliament and of the Council on undesirable substances in animal feed.

The test material used in this exercise was a commercial mineral feed for piglets provided by AGES, Zentrum Analytik und Mikrobiologie, in Austria. The material, naturally contaminated, was processed, bottled, labelled and dispatched by the Reference Materials Unit of the IRMM. The samples were dispatched on the second half of October 2008. Each participant received one bottle containing approximately 30 g of test material. Thirty-one participants from 25 countries registered to the exercise of which 28 submitted results for total Cd and for total Pb, 22 submitted results for total As and 27 submitted results for extractable Cd and for extractable Pb. Tow laboratories did not submit results due to a break down in the instruments that were to be used for the analyses.

The assigned values (Xref) for total and extractable Cd and Pb were provided by IRMM using isotope dilution-inductively coupled plasma-mass spectrometry (ID-ICP-MS). The analytical uncertainty of Xref, uchar, was calculated according to the ISO Guide to the Expression of Uncertainty in Measurement (GUM). The assigned value for total arsenic was provided by the Studiecentrum voor Kernenergie (SCK) using neutron activation analysis. The analytical uncertainty of Xref, uchar, for total arsenic was calculated according to GUM and was based on the spreadsheet technique developed by Kragten. Homogeneity and stability studies were subcontracted to Bayer Antwerpen. The uncertainties of the respective assigned values, uref, were calculated combining the analytical uncertainty, uchar, with a contribution for the between-bottle homogeneity, ubb, and for the short term stability of the test material, usts. Participants were invited to report the uncertainty of their measurements. This was done by 25 laboratories for total Cd, 24 laboratories for total Pb, 18 laboratories for total As, 22 laboratories for extractable Cd and 21 laboratories for extractable Pb.

Laboratory results were rated with z and zeta scores in accordance with ISO 13528. The standard deviations for proficiency assessment (also called target standard deviation) were calculated using the modified Horwitz equation and were: 15 % of the assigned value for total and extractable Cd, 15.5 % of the assigned value for total and extractable Pb and 16 % of the assigned value for total As.

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